Targeting Treg-expressed STAT3 enhances NK-mediated surveillance of metastasis and improves therapeutic response in pancreatic adenocarcinoma

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Background

- PDAC has a low five-year survival rate of 9%.[1]
- PDAC is resistant to conventional and targeted therapies. Its immunosuppressive tumor microenvironment (TME) plays a large role.[2]
- TME of PDAC is devoid of cytotoxic effector immune cells like NK, CD4, and CD8 cells and contains many immunosuppressive cells including Tregs.[3-4]
- Stereotactic body radiation therapy (SBRT), used to invigorate the body's immune response, showed inferior results to standard of care in a recent clinical trial.[5]
- Analysis of patient specimens before and after SBRT treatment showed an over-expressed STAT3 signal within the NK and Treg immune cell subsets.
- Stat3 is known to be activated in PDAC; its expression correlates with tumor grade.[6]

Question: What is the mechanisms by which STAT3 signaling on immune subsets affects their cytotoxic, suppressive, or surveillance potential and how this regulates local growth and systemic dissemination in context of radiation therapy?

Hypothesis: Sustained activation of STAT3 within the NK and Treg compartments hinders SBRT potency and promotes metastasis and disease spread in PDAC

Methods

- For local orthotopic implantations mouse pancreata were injected with 200,000 KPC cells suspended 1:1 in Matrigel.
- For metastatic orthotopic implantations spleens were first ligated with horizon clips and 1 hemispleen was injected with 200,000 KPC cells suspended in 50ul 10% RPMI followed by washout injection of 50µl PBS. Splenic vessels were then ligated with horizon clips and hemispleen was excised prior to closure.
- Murine STAT3 ASO or control ASO were dosed at 50mg/kg 3 times per week intraperitoneally starting at 1 day prior to RT and maintained for the duration of the study. Diphtheria toxin was dosed at 50µg twice per week beginning one day prior to tumor implantation. αNK1.1 antibody was administered at a dose of 200µg twice per week beginning one day prior to implantation.
- Flow cytometric analysis run on Cytek Aurora Spectral Cytometer

Results

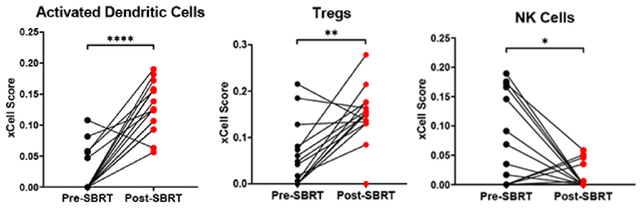

Figure 1: Paired sequencing of PDAC tumors before and after treatment with SBRT showing changes in the frequency of activated dendritic cells, Tregs, and NK cells.

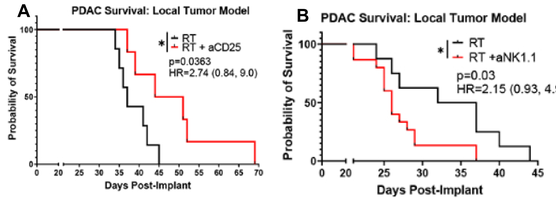

Figure 2: Kaplan-Meier survival analysis of wildtype mice implanted with local orthotopic pancreatic tumors treated with RT and (a) CD25 Treg depleting antibody and (b) αNK1.1 NK cell depleting antibody.

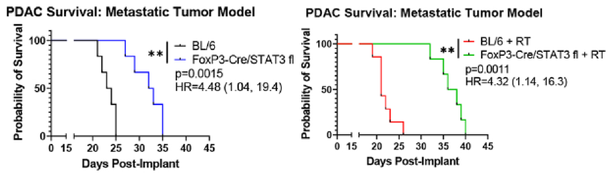

Figure 3: Kaplan-Meier survival analysis of wildtype and FoxP3-Cre/STAT3 fl mice implanted with local orthotopic pancreatic tumors without and with RT.

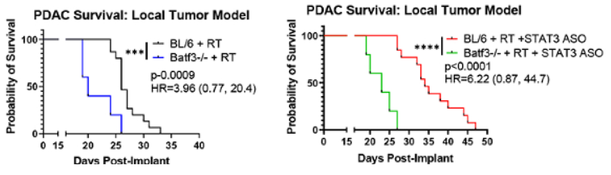

Figure 4: Kaplan-Meier survival analysis of wildtype C57BL/6 and CD8a+ DCs knock out mice implanted with local orthotopic pancreatic tumors.

*indicates P<0.05, **<0.01, ***<0.001

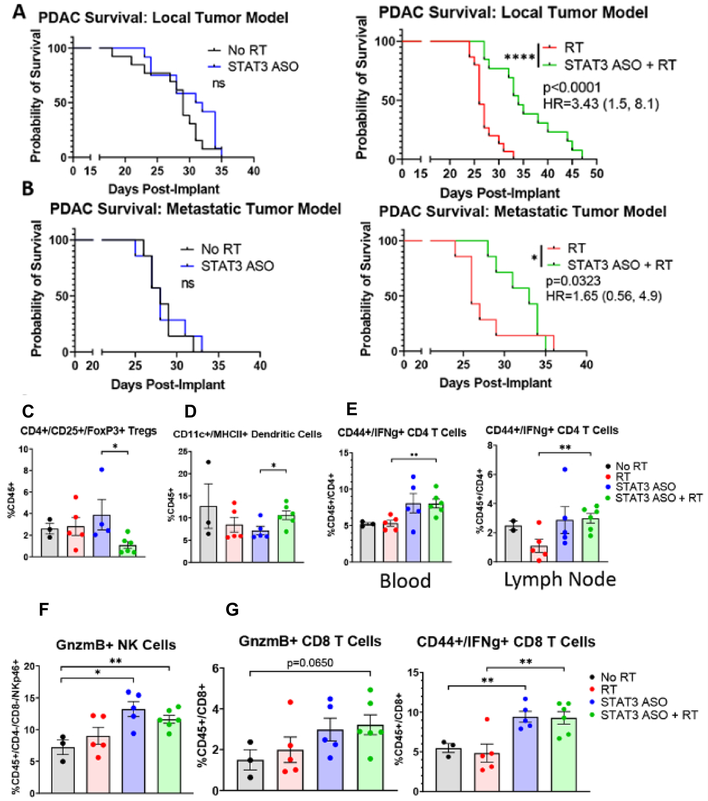

Figure 5: Kaplan-Meier survival analysis of wildtype C57BL/6 mice bearing local orthotopic (a) and metastatic (b) tumors treated with and without STAT3 ASO and RT. Flow cytometry analysis in wildtype C57BL/6 tumor bearing mice following STAT3 ASO and RT treatment of tumor-infiltrating Tregs (c) and dendritic cells (d), (e) CD4 T cells in the circulating blood (left) and lymph nodes (right), (f) GnzmB+ NK cells in the circulating blood, and (j) CD8 T cell activation in the circulating blood defined by GnzmB positivity (left) and as CD44/IFNg co-positivity (right).

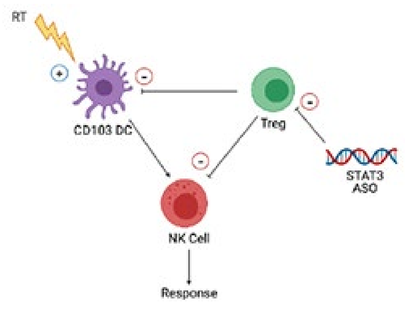

Figure 6: Model of interactions between immune populations.

Conclusions

- Tregs are a core element of therapeutic resistance in pancreatic cancer, and response to RT is only enhanced when Tregs are depleted or the target of inhibition.
- Systemic delivery of a STAT3 anti-sense oligonucleotide (ASO) in combination with SBRT results in tumor growth regression, decreased metastasis, and reversal of immunosuppression.
- A lack of NK cells confers decreased survival in PDAC, while suppression of Tregs leads to increased survival. Dendritic cells are an important mediator, as STAT3 ASO therapy response is eliminated without DCs.
- Combination therapies such as STAT3 ASO with RT may improve response to treatment in pancreatic cancer.

Disclosures

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References

1. Siegel RL; Miller KD; Jemal A, Cancer statistics, 2020. CA Cancer J Clin 2020, 70 (1), 7–30.
2. Sahin IH; Elias H; Chou JF; Capanu M; O'Reilly EM, Pancreatic adenocarcinoma: insights into patterns of recurrence and disease behavior. BMC Cancer 2018, 18 (1), 769.
3. Amedei A; Niccolai E; Benagiano M; Della Bella C; Cianchi F; Bechi P; Taddei A; Bencini L; Farsi M; Cappello P; Prisco D; Novelli F; D'Elios MM, Ex vivo analysis of pancreatic cancer-infiltrating T lymphocytes reveals that ENO-specific Tregs accumulate in tumor tissue and inhibit Th1/Th17 effector cell functions. Cancer Immunol Immunother 2013, 62 (7), 1249–60.
4. Zhao F; Obermann S; von Wasielewski R; Haile L; Manns MP; Korangy F; Greten TF, Increase in frequency of myeloid-derived suppressor cells in mice with spontaneous pancreatic carcinoma. Immunology 2009, 128 (1), 141–9.
5. Katz MHG; Shi Q; Meyers JP; Herman JM; Choung M; Wolpin BM; Ahmad S; Marsh R. d. W.; Schwartz LH; Behr S; Frankel WL; Collisson EA; Leenstra JL; Williams TM; Vaccaro GM; Venook AP; Meyerhardt JA; O'Reilly EM, Alliance A021501: Preoperative mFOLFIRINOX or mFOLFIRINOX plus hypofractionated radiation therapy (RT) for borderline resectable (BR) adenocarcinoma of the pancreas. Journal of Clinical Oncology 2021, 39 (3_suppl), 377–377.
6. Lesina M; Kurkowski MU; Ludes K; Rose-John S; Treiber M; Klöppel G; Yoshimura A; Reindl W; Sipos B; Akira S; Schmid RM; Algül H, Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. Cancer Cell 2011, 19 (4), 456–69.